P1 1081468

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

October 21, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/424,978*
FILING DATE: *November 08, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/28742*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. R. GRANT
Certifying Officer

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

11-12-02 [illegible]

Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.	ET899942962US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Hartmut M.	Hanauske-Abel	Edgewater, New Jersey
Anthony	Popowicz	New York, New York
Edith	Wolff	Bethesda, Maryland

☑ *Additional inventors are being named on the* 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Small Molecule Ligands Of Eukaryotic Translation Initiation Factor 5A (eIF-5A) and Their Use to Control Proliferation Of Cells.

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number 33348 → *Place Customer Number Bar Code Label here*

OR *Type Customer Number here*

☐ Firm or Individual Name	Margaret Mary Kozik Richardson				
Address	UMDNJ - Office of Patents and Licensing				
Address	335 George Street, Suite 3200				
City	New Brunswick	State	New Jersey	ZIP	08901
Country	US	Telephone	732-235-9350	Fax	732-235-9358

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages*	4	☐ CD(s), Number	
☑ Drawing(s) *Number of Sheets*	7	☑ Other (specify)	Claims (1 pg); Post Card
☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑	Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE AMOUNT ($)
☐	A check or money order is enclosed to cover the filing fees	
☑	The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 500756	$80.00
☐	Payment by credit card. Form PTO-2038 is attached.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☑ Yes, the name of the U.S. Government agency and the Government contract number are: NIH Grant Number: R01CA42908

Respectfully submitted,

SIGNATURE [signature]

Date 11/08/2002

TYPED or PRINTED NAME Margaret Mary Kozik Richardson

REGISTRATION NO. *(if appropriate)* 47,023

Docket Number: NJMS-02-90

TELEPHONE 732-235-9350

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SB/16 (02-01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Docket Number	NJMS-02-90

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Paul M. J.	Clement	Bethesda, Maryland
Myung-Hee	Park	Bethesda, Maryland
Bernadette M.	Cracciolo	Edgewater, New Jersey

Number 2 of 2

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE TRANSMITTAL for FY 2002

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete If Known	
Application Number	
Filing Date	Herewith
First Named Inventor	HANAUSKE-ABEL, Hartmut M.
Examiner Name	
Group Art Unit	
Attorney Docket No.	NJMS-02-90

METHOD OF PAYMENT

1. ☑ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
   - Deposit Account Number: 500756
   - Deposit Account Name: University of Medicine and Dentistry of New Jersey
   - ☑ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
   - ☑ Applicant claims small entity status. See 37 CFR 1.27
2. ☐ Payment Enclosed: ☐ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	80.00

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=	
Independent Claims		- 3** =		X	=	
Multiple Dependent					=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Margaret Mary Kozik Richardson	Registration No. (Attorney/Agent)	47,023	Telephone	732-235-9350
Signature	*Margaret Richardson*			Date	11/08/2002

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	None assigned	**Docket No.**	:	NJMS-02-90
Examiner	:	None assigned	**Dated**	:	November 8, 2002
Serial No.	:	Unknown			
Filed	:	Herewith			
Inventors	:	Hartmut M. Hanauske-Abel, et al.			
Title	:	"Small Molecule Ligands Of Eukaryotic Translation Initiation Factor 5A (eIF-5A) And Their Use to Control Proliferation Of Cells"			

Assistant Commissioner of Patents
Washington, DC 20231
BOX PROVISIONAL PATENT APPLICATION

Sir:

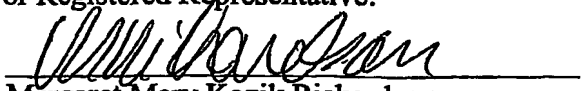
Certificate of Express Mailing Under 37 CFR 1.10

For
Provisional patent application consisting of:
Specification (4 pages);
Claims (1 pages);
Drawings (7 sheets)
Provisional Patent Application Cover Sheet (1 page);
Certificate of Mailing by Express Mail;
Fee Transmittal Sheet (in Duplicate); and
Postcard

I hereby certify that the accompanying correspondence is being deposited with the "Express Mail Post Office to Addressee" service of the United States Postal Service in an envelope addressed to Assistant Commissioner for Patents, Patent and Trademark Office, Box PROVISIONAL PATENT APPLICATION, Washington, D.C. on the date indicated below. The number of the "Express Mail" mailing label **ET899942962US** has been placed on the accompanying correspondence prior to mailing. It is therefore respectfully requested that this correspondence be considered as having been filed in the Office on the date shown below in accordance with the provisions of 37 C.F.R. §1.10.

Name of Applicant, Assignee, Applicant's Attorney or Registered Representative:

Margaret Mary Kozik Richardson, Esq.
UMDNJ, Office of Patents and Licensing
335 George Street, Suite 3200
P.O. Box 2688
New Brunswick, New Jersey 08903-2688
Phone: 732.235.9350 Fax: 732.235.9358



Margaret Mary Kozik Richardson
Registration No. 47,023

November 8, 2002
Date

Small Molecule Ligands Of Eukaryotic Translation Initiation Factor 5A (eIF-5A) And Their Use to Control Proliferation Of Cells.

The government has certain rights in this invention.

Abstract

We have identified eukaryotic translation initiation factor 5A (eIF-5A) as a non-enzymatic target for folate-derived agents that interfere with cell proliferation.

Background

The eukaryotic translation initiation factor 5A (eIF-5A), which exists in two genetically distinct variants, I and II, is involved in the translational control of gene expression. Compounds that affect the physiological structure of eIF-5A, in particular its lysine-derived hypusine residue, cause a unique subset of cellular mRNA's termed *hymns* (hypusine-dependent messenger nucleic acids) to disappear from cellular polysomes (1). This effect is associated with arrest in the late G1 phase of the cell cycle, immediately before the initiation of DNA replication (2).

Folate, a vitamin required for metabolism of one-carbon units and essential for the biosynthesis of DNA and RNA building blocks, is also known to be involved in the translational control of gene expression. For instance, whereas the levels of the mRNA encoding dihydrofolate reductase or of the mRNA encoding the folate receptor alpha do not change in response to alterations of folate levels, the translation of these mRNAs occur in a manner that is sensitive to the concentration of folate or folate analogs and antagonists (3,4).

Surprisingly, we noted that the eIF-5As display significant structural homologies with at least one of the proteins whose translational efficiency is affected by folate/antifolate levels: the dihydrofolate reductases (DHFRs).

Recent structural analyses of eIF-5A indicate that its C-terminal part folds like the cold-shock protein A of *E.coli*, (5), and that its most N-terminal part contains motifs II, III, IV, and V of ATP-utilizing mRNA helicases, required for unwinding of mRNA duplexes (6). Using the spatial coordinates of only the N-terminal part of eIF-5A of *M. jannaschii* (PDB# 1EIF), we noted a significant homology with the crystal structure of plasmid-encoded DHFR of *E. coli* (PDB# 1vie), using the Dali algorithm (Z score = 4.4)., **See Figure 1**

Similarly, optimized sequence alignment between human DHFR (Acc.# XM_165390) and the human eIF-5As (**I**: Acc.# NP_001961; **II**: Acc.# NP_065123) revealed 37% identify/similarity with eIF-5A-I and 35% identify/similarity with eIF-5A-II. The N-terminal domain of the human eIF-5As displays several isolocated residues that in DHFR participate in binding of folate and the antifolate methotrexate (e.g. Ile^{7}, Pro^{61}, Arg^{70}), and of NADPH (e.g. Gly^{20}, Lys^{54},Gly^{117},Ser^{118}). Distinct sequence differences affecting residues involved in catalytic efficiency, such as the E30Q isolocation, suggest the eIF-5As display limited if any DHFR activity, **see Figure 2.**

Based on the above background, we hypothesize that in eIF-5A, the DHFR-like structural motifs function in rendering the translational control of *hymns* sensitive to levels of folate and its analogs. The discovery within eIF-5As of substructures that are a prerequisite for binding of folate and of folate derivatives identifies the eIF-5As as potential targets for these small molecule ligands, and points to multiple applications in conditions that involve cell proliferation.

Detailed Description of the Invention

It is established that eIF-5A is directly involved in the cellular machinery that controls the onset of DNA replication and initiates proliferation of cells.

Surprisingly, we noted marked similarities between eIF-5A and dihydrofolate reductase at the level of their primary as well as their tertiary structures, involving in particular motifs that in the enzyme enable the binding of folate and its derivatives. eIF-5A and dihydrofolate reductase are both known to control translational efficiancy of specific mRNAs, the latter in a manner controlled by the level of folate/antifolate.

For those knowledgeable in the art, the outlined results represent information directly enabling the use of folate and its derivatives, and the discovery and development of novel folate analogs, that modulate the translational control of gene expression executed by eIF-5A.

Examples

The examples are not intended to limit the invention.

Example 1: The Crystal Structure

Reactivation of DOHH causes rapid reappearance of *hymns* at polysomes and subsequent, highly synchronized entry of cells into S phase. The *hymns*, though encoding very diverse cell cycle-relevant proteins, share common nucleotide motifs, termed JSBs, in their untranslated 3'- and 5'-regions. Recent structural analyses of eIF-5A indicate that its C-terminal part folds like the cold-shock protein A of *E. coli*, which prevents mRNA duplex formation at low temperatures (*Structure* 6, 1207; 1998), and that human eIF-5As in their most N-terminal part contain motifs II, III, IV, and V of ATP-utilizing mRNA helicases, required for unwinding of mRNA duplexes (FASEB J 16, A549; 2002). Hypothesizing that eIF-5A crystal structure / sequence data contain further clues for its interaction with specific mRNAs essential for cell cycle control, we refined parameters and strategies of an exhaustive database analysis. Using the spatial coordinates of only the N-terminal part of eIF-5A of *M. jannaschii* (PDB# 1EIF), we noted a significant homology with the crystal structure of plasmid-encoded dihydrofolate reductase (DHFR) of *E. coli* (PDB# 1vie), using the Dali algorithm (Z score = 4.4), **see Figure 1.**

Example 2: Sequence Isolation

Optimized sequence alignment between human DHFR (Acc.# XM_165390) and the human eIF-5As (**I**: Acc.# NP_001961; **II**: Acc.# NP_065123) revealed 37% identify/similarity with eIF-5A-**I** and 35% identify/similarity with eIF-5A-**II**. The N-terminal domain of the human eIF-5As displays

several isolocated residues that in DHFR participate in binding of folate and methotrexate (e.g. Ile7, Pro61, Arg70) and of NADPH (e.g. Gly20, Lys54,Gly117,Ser118), whereas distinct differences, such as the E30Q isolocation, suggest limited DHFR activity, **see Figure 2.Example 3: Antibody Characterization**

The eukaryotic translation initiation factor 5A (eIF-5A) exists in two genetically distinct variants, **I** and **II**. Both contain a single hypusine residue, formed by enzymatic hydroxylation within a -Gly-X-Y-Gly- collagen motif known to fold into a ß-turn. Like the collagens, the eIF-5As are subject to posttranslational protein hydroxylation by a 2-oxoacid-utilizing dioxygenase; the eIF-5A-hydroxylating enzyme is the hypusine-forming deoxyhypusyl hydroxylase (DOHH). The catalytic cycle of all 2-oxoacid-utilizing dioxygenases including DOHH, follows a unique pathway formulated by Hanauske-Abel and Günzler (1), which proceeds at their non-heme ferrous ion as a ligand reaction between a chelating 2-oxoacid moiety and an end-on coordinated dioxygen unit to generate the reactive iron-oxo species essential for product formation. The detailed orbital interactions specified by the HAG mechanism were essential for the rational discovery of inhibitors that suppress hypusine formation in eIF-5As, revealing an essential role in polysomal loading of specific mRNAs (*hymns*) and in the onset of DNA replication, i.e. exit from G1 (2,3). ***Figure 3 shows*** the typical effect of a hypusine inhibitor on cellular DOHH activity (blue) and on cell cycle progression (red / black) [reproduced from ref. 3]. Thus, the eIF-5As and their unique hypusine residue appear closely related to, or even represent the molecular equivalent of, one of the major restriction points in the cell cycle: the irreversible commitment to initiate DNA replication. The hypusine domain of eIF-5A has emerged as the target for several experimental cytostatic agents and for antiproliferative drugs already in clinical use, among them the antifungal compound ciclopirox (4,5).

We recently characterized an antibody, NIH-353, as being reactive with human eIF-5A only if hypusine has been formed (***Figure 4A***). The hypusine residue is not genetically encoded; rather, it derives from a genetically encoded lysine moiety, after butylamine transfer utilizing spermidine, followed by the DOHH-mediated hydroxylation utilizing atmospheric oxygen (***Figure 4B***). Immunohistochemical analysis of human tissues and cancers has confirmed that the hypusine domain-selective NIH-353 labels only proliferating cells, as shown in ***Figure 4C*** for normal endometrium (proliferative phase) [compare with Ki67 control stain, ***Figure 4D***].

Material and Methods

Protein structural alignments used the crystal coordinates of eIF-5A from *M.jannaschii* (PDB# 2EIF), the plasmid-encoded dihydrofolate reductase of *E.coli* (PDB# 1VIE and PDB#1VIF), and the cold-shock protein of *E.coli* (PDB# 1MJC). Comparisons were performed with Dali v. 2. Molecules were visualized with InsightII. Linear alignments were generated with ClustalW and manually optimized.

Figures

One-dimensional (A,B) and three-dimensional (C) alignments of the N-terminal and the C-terminal part of archaeal eIF-5A with bacterial dihydrofolate reductase (bDHFR) and with bacterial cold shock protein A (csp-A).

The similarity between csp-A and the C-terminal part of eIF-5A has been observed before (7).

The N-terminal part of eIF-5A contains the lysine precursor of hypusine (A) and the ß-turn of the hypusine domain (C), both highlighted in blue.

Note the red arrows in the linear (A) and in the steric (C) alignments. The bDHFR residue Gln51, whose planar terminal amide group sandwiches onto the the planar pterin moiety of folate (PDB #1vif), is iso-positioned with the eIF-5A residue Arg44, which also displays a similarly oriented, planar guanidinium group.

Figure 4A: Western blot of antibody

Figure 4B: Pathway

Figures 4C and 4D: Immunohistochemical analysis of human tissues and cancers

References

1. Hanauske-Abel, H. M., Slowinska, B., Zagulska, S., Wilson, R.C., Staiano-Coico, L., Hanauske, A.-R., McCaffrey, T., Szabo, P. (1995)
Detection of a sub-set of polysomal mRNAs associated with modulation of hypusine formation at the G1-S boundary. Proposal of a role for eIF-5A in onset of DNA replication.
FEBS Lett. **366**, 92-98.

2. Hanauske-Abel, H. M., Park, M.-H., Hanauske, A.-R., Popowicz, A.M., Lalande, M., Folk, J.E. (1994)
Inhibition of the G1-S transition of the cell cycle by inhibitors of deoxyhypusine hydroxylation.
Biochim. Biophys. Acta **1221**, 115 -124 .

3. Ercikan-Abali EA; Banerjee D; Waltham MC; Skacel N; Scotto KW; Bertino JR (1997)
Dihydrofolate reductase protein inhibits its own translation by binding to dihydrofolate reductase mRNA sequences within the coding region.
Biochemistry **36**, 12317 - 12322

4. Zhu WY; Alliegro MA; Melera PW (2001)
The rate of folate receptor alpha (FR alpha) synthesis in folate depleted CHL cells is regulated by a translational mechanism sensitive to media folate levels, while stable overexpression of its mRNA is mediated by gene amplification and an increase in transcript half-life.
J Cell Biochem **81**, 205 – 219

5. Peat TS; Newman J; Waldo GS; Berendzen J; Terwilliger TC (1998)
Structure of translation initiation factor 5A from Pyrobaculum aerophilum at 1.75 A resolution.
Structure 6, 1207-1214

6. HM Hanauske-Abel, A R Hanauske, B Slowinska, A M Popowicz (2002)
The cell cycle-controlling protein eIF-5A : Identification of functional regions relevant for interaction with its mRNA partners.
FASEB J. 16, A549

Claims

We claim the following :

1. A ligand specific for the folate-binding domain of eukaryotic translation initiation factor 5A (eIF-5A), said folate-binding domain being defined as composed of residue motifs common to eIF-5A and to the bacterial and human dihydrofolate reductases as shown in Figure 2, and said ligand being characterized as binding to the folate-binding domain of eIF-5A.

2. A ligand as claimed in Claim 1 in which the ligand is an analog of folate, or a derivative or fragment thereof, which specifically binds to an eIF-5A molecule only if said eIF-5A contains a folate-binding domain, and if eIF-5A lacks said domain does not bind.

3. A method for identifying a novel clss of folate derivatives that are inhibitors of proliferation yet do not inhibit folate-dependent enzymes.

4. A method for independent inhibiting in a cell the biological activity of the folate-binding domain of eIF-5A as composed of residue motifs identified in Figure 2, comprising introducing into said cell a low-molecular weight molecule that binds to the folate-binding domain of eIF-5A to thereby inhibit the biological activity of eIF-5A in the translational control of gene expression required for cell proliferation.

Figure 1

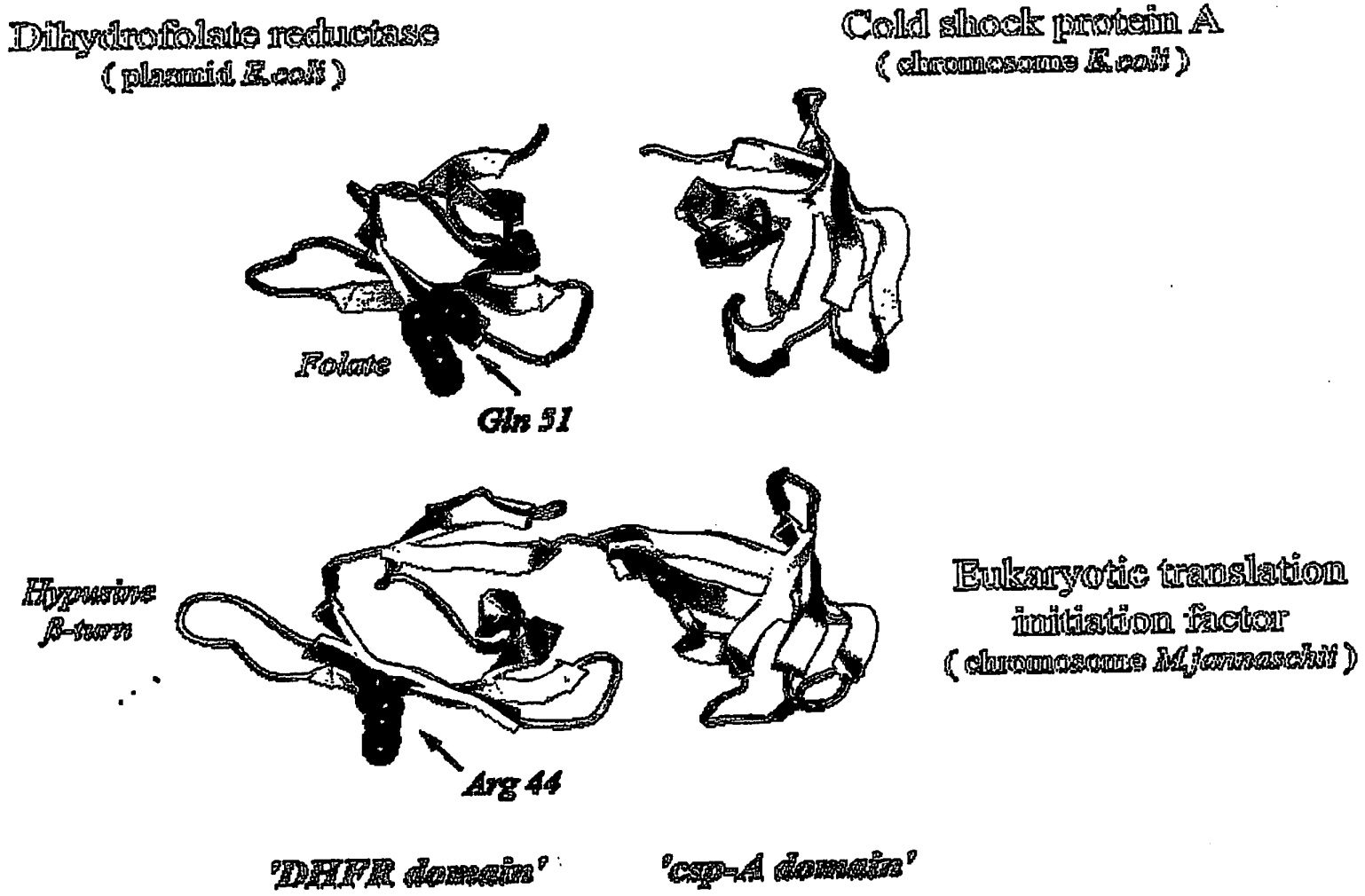
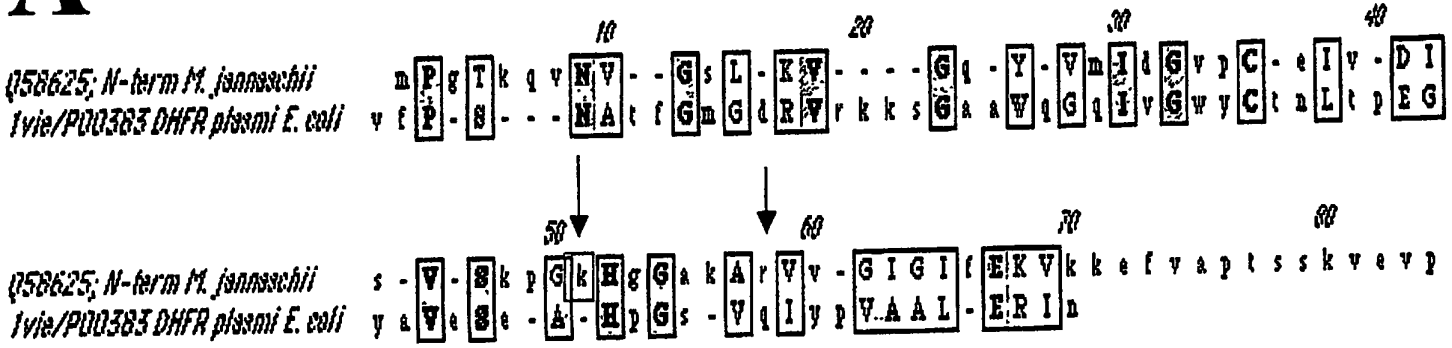

Dihydrofolate reductase
(plasmid E.coli)
Cold shock protein A
(chromosome E.coli)
Folate
Gln 51
Hypusine
β-turn
Arg 44
Eukaryotic translation
initiation factor
(chromosome Mjannaschii)
'DHFR domain'
'csp-A domain'

Figure 2

A


Q58625; N-term M. jannaschii
1vie/P00383 DHFR plasmi E. coli
Q58625; N-term M. jannaschii
1vie/P00383 DHFR plasmi E. coli

B

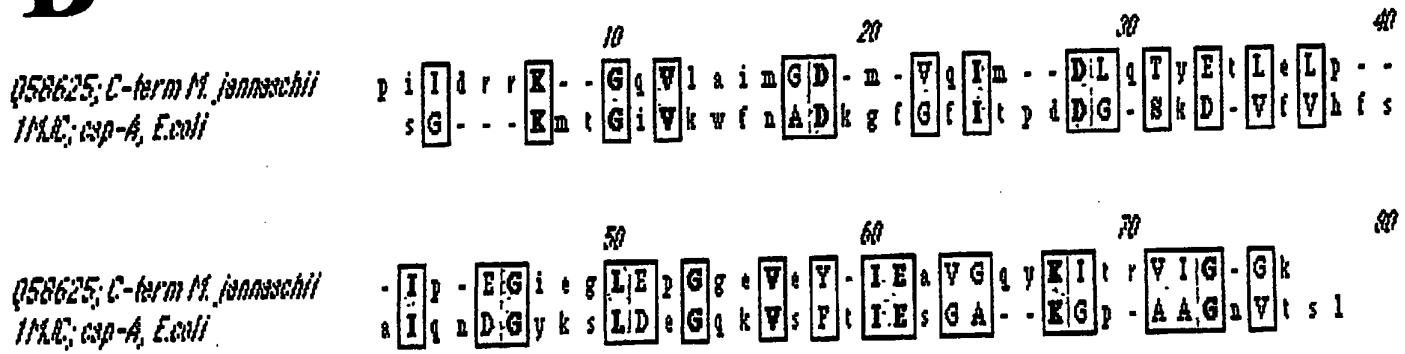

Q58625; C-term M. jannaschii
1MJC; csp-A, E.coli
Q58625; C-term M. jannaschii
1MJC; csp-A, E.coli

Figure 3

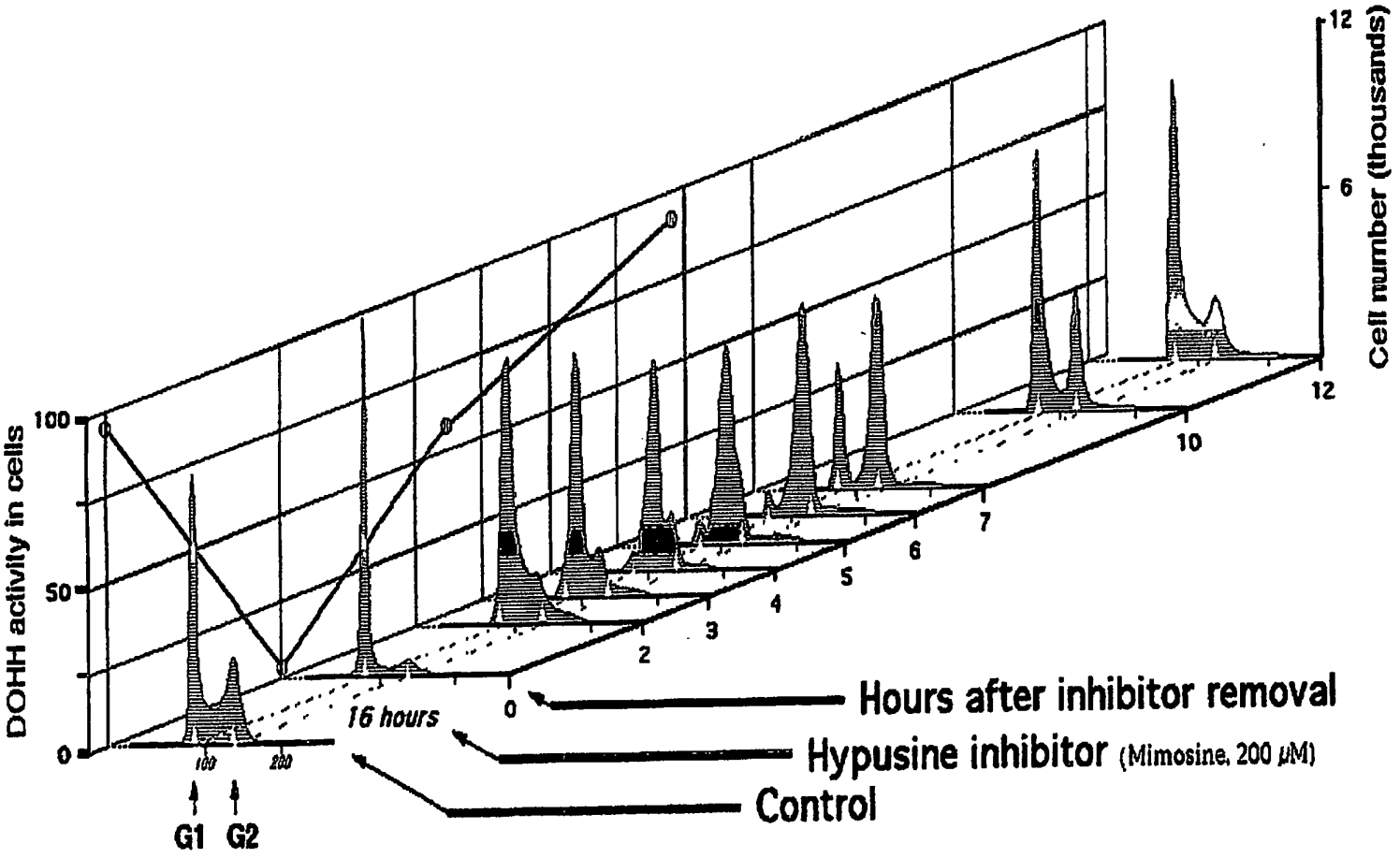

DOHH activity in cells
100
50
0
Cell number (thousands)
12
6
12
10
7
6
5
4
3
2
0
16 hours
100
200
G1
G2
Hours after inhibitor removal
Hypusine inhibitor (Mimosine, 200 μM)
Control

Figure 4A

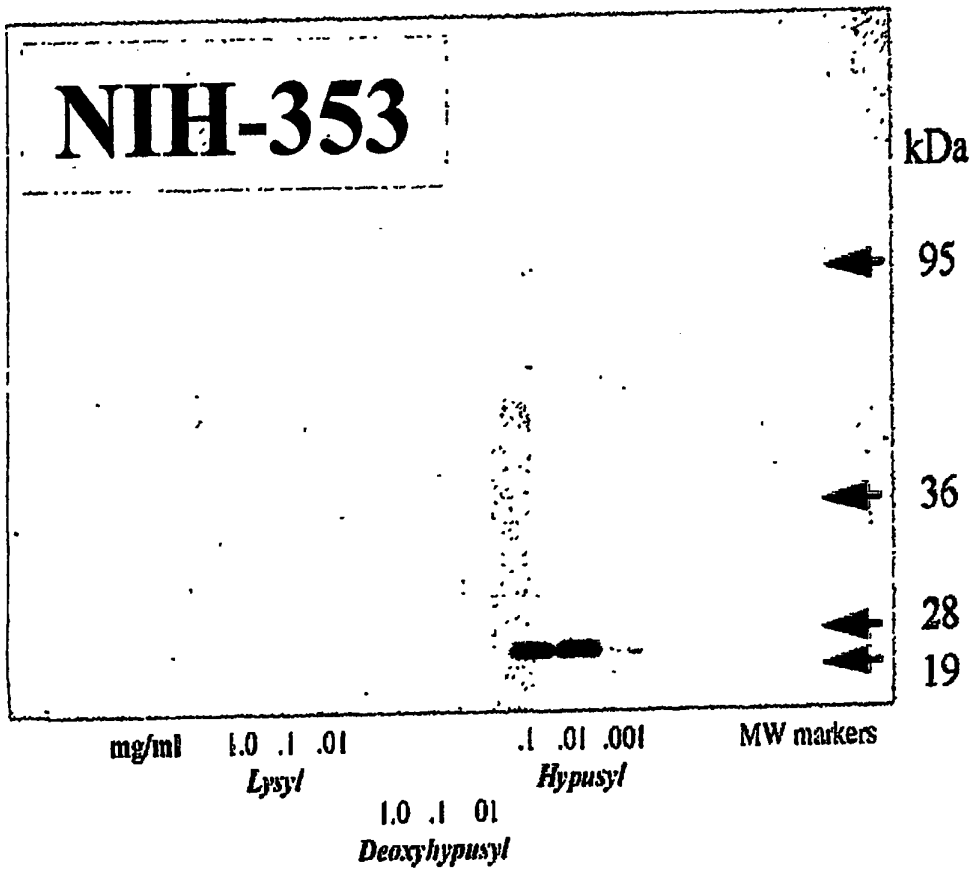

NIH-353
kDa
95
36
28
19
mg/ml 1.0 .1 .01
Lysyl
.1 .01 .001
Hypusyl
MW markers
1.0 .1 01
Deoxyhypusyl

Figure 4B

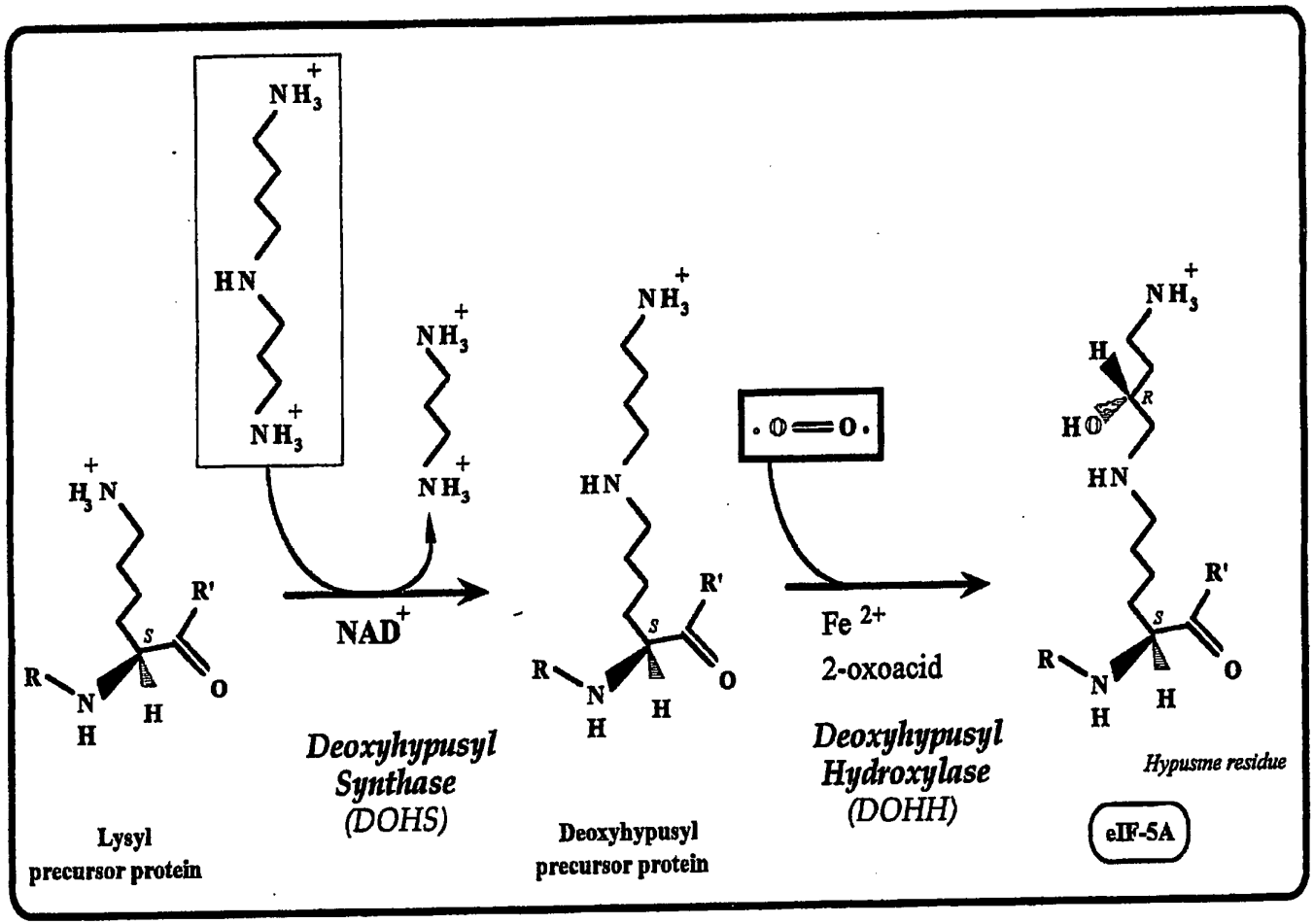

NAD+
Deoxyhypusyl Synthase (DOHS)
Lysyl precursor protein
Deoxyhypusyl precursor protein
Fe 2+
2-oxoacid
Deoxyhypusyl Hydroxylase (DOHH)
Hypusine residue
eIF-5A

Figure 4C

NIH-353

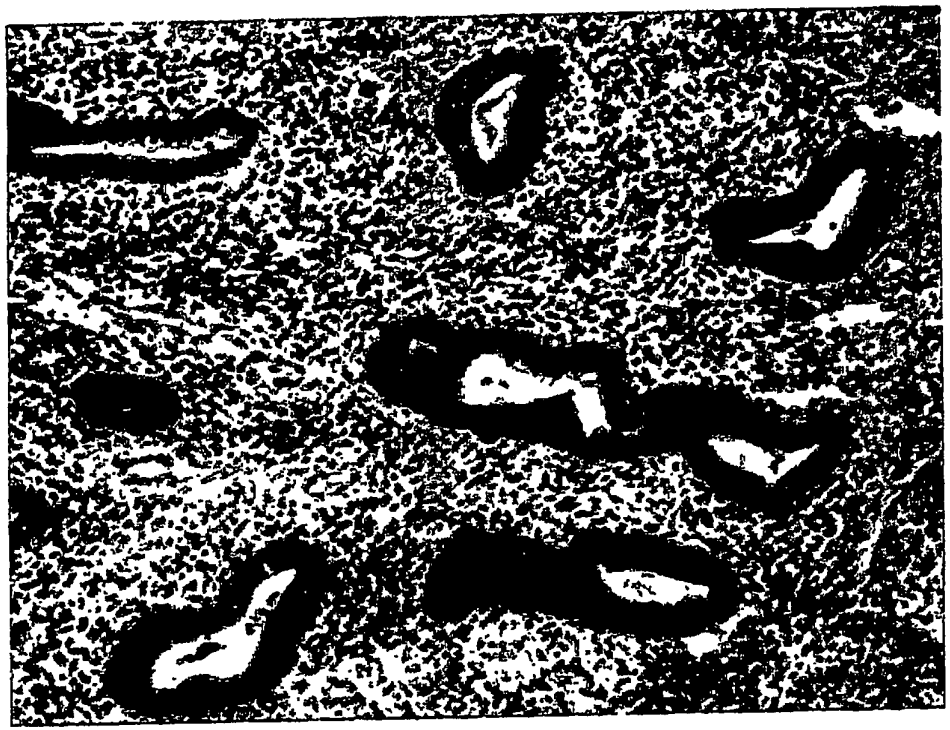


Figure 4D

Ki-67 control